Departement für Pferde
Klinik für Pferdechirurgie
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. med. vet. Anton Fürst

An in vitro investigation of PRP-gel as a cell and growth factor delivery vehicle for tissue engineering

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Jagoda Jalowiec

Tierärztin
von Debica, Polen

genehmigt auf Antrag von

Prof. em. Dr. med. vet. Dr. h. c. Jörg A. Auer, Referent

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“An in vitro investigation of PRP-gel as a cell and growth factor delivery vehicle for tissue engineering”

AutorInnenschaft: Jalowiec JM, D’Este M, Bara JJ, Denom J, Menzel U, Alini M, Verrier S, Herrmann M

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CV

Abstract EN

Platelet rich plasma (PRP) has been used for different applications in human and veterinary medicine. Studies have shown promising therapeutic effects of PRP; however there are still many controversies regarding its composition, properties and clinical efficacy. The aim of this study was to evaluate the influence of different platelet concentrations on the properties and growth factor release profile of PRP-gels. Also, the viability of incorporated bone marrow-derived human mesenchymal stem cells (MSCs) was investigated. PRP was prepared from human platelet concentrates. Viscoelastic properties of PRP-gels were evaluated by rheological studies. The release of GFs and inflammatory proteins was measured using ELISA. MSC viability and proliferation in PRP-gels was assessed over 7 days by cell viability staining. Cell proliferation was examined using DNA quantification. All tested PRP-gels showed effective crosslinking. A positive correlation between protein release and the platelet concentration was observed. The chemokine CCL5 was the most abundant. The greatest release appeared within the first hours after gelification. MSCs could be successfully cultured in PRP-gels over 7 days. The results suggest that PRP-gels represent a suitable carrier for cell- and growth factor delivery for tissue engineering. Lowest platelet concentration provides the most favorable environment for MSCs. The platelet concentration is an important consideration for the clinical application of PRP-gels.

(Platelet Rich Plasma, Stem cells, PRP-gel)

Zusammenfassung DE

Plättchen-reiches Plasma (PRP) wird in vielen Bereichen der Medizin verwendet. Bisherige Studien zeigten unterschiedliche Resultate bezüglich der Effizienz und der Eigenschaften von PRP. Ziel dieser Arbeit war die Evaluierung des Effekts verschiedener Plättchenkonzentrationen auf die Freisetzung von Wachstumsfaktoren und Eigenschaften von PRP-Gelen. Zusätzlich wurde die Viabilität von mesenchymalen Stammzellen (MSCs) in PRP-Gelen untersucht. PRP wurde aus Thrombozytenkonzentraten gewonnen. Die viskoelastischen Eigenschaften der PRP-Gele wurden mittels rheologischer Studien evaluiert. Die Freisetzung der Wachstumsfaktoren und Entzündungsmediatoren wurde mit ELISA getestet. Die Zellviabilität in PRP-Gelen wurde mit Vitalfärbung evaluiert. Die Zellproliferation wurde anhand einer DNA Quantifizierung getestet.Alle PRP-Gele zeigten eine erfolgreiche Vernetzung. Eine positive Korrelation zwischen der Proteinfreisetzung und der Plättchenkonzentration konnte für alle Versuchszeitpunkte nachgewiesen werden. CCL5 in der höchsten Konzentration gefunden und die grösste Freisetzung in den ersten Stunden nach der Gelierung detektiert wurde. Die MSCs über einen Zeitraum von 7 Tagen effektiv in PRP-Gelen gezüchtet werden können. Die PRP-Gele können als ein Zell- und Wachstumsfaktorenträger verwendet werden. Die niedrigste Plättchenkonzentration in PRP-Gelen zeigte die besten Resultate bezüglich der Zellviabilität und Proliferation.

(Plättchen-reiches Plasma, Stamzellen, PRP-Gel)

An in vitro investigation of PRP-gel as a cell and growth factor delivery vehicle for tissue engineering

Jagoda M Jalowiec[1], Matteo D'Este PhD[1], Jennifer J Bara PhD[1], Jessica Denom[2], Ursula Menzel PhD[1], Mauro Alini PhD[1], Sophie Verrier PhD[1*], Marietta Herrmann PhD[1]

[1]AO Research Institute Davos, Davos, Switzerland

[2]Biologie Fonctionnelle et Adaptative, CNRS UMR 8251, Université Paris Diderot Paris 7, Paris, France

Author contact information:

Jagoda M. Jalowiec AO Research Institute, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland; phone: +41 81 414 22 11; fax: +41 81 414 22 88; jalowiec.jagoda@gmail.com

Matteo D'Este AO Research Institute, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland; phone: +41 81 414 24 53; fax: +41 81 414 22 88; matteo.deste@aofoundation.org

Jennifer J. Bara AO Research Institute, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland; phone: +41 81 414 24 81; fax: +41 81 414 22 88; jennifer.bara@aofoundation.org

Jessica Denom Université Paris Diderot Paris 7, CNRS UMR 8251, Unité BFA - Equipe REGLYS : Régulation Centrale de la Glycémie, Bâtiment Buffon, case courrier 7126, pièce 504A, 4 rue Marie-Andrée Lagroua Weill Halle, 75205 Paris cedex 13 phone: +33 01 57 27 77 89; jessica.denom@univ-paris-diderot.fr

Ursula Menzel AO Research Institute, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland; phone: +41 81 414 24 82; fax: +41 81 414 22 88; ursula.menzel@aofoundation.org

Mauro Alini AO Research Institute, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland; phone: +41 81 414 23 10; fax: +41 81 414 22 88; mauro.alini@aofoundation.org

Sophie Verrier* AO Research Institute, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland; phone: +41 81 414 24 48; fax: +41 81 414 22 88; sophie.verrier@aofoundation.org

Marietta Herrmann AO Research Institute, Clavadelerstrasse 8, 7270 Davos Platz, Switzerland; phone: +41 81 414 23 18; marietta.herrmann@aofoundation.org

*** corresponding author**

ABSTRACT

Platelet rich plasma (PRP) has been used for different applications in human and veterinary medicine. Many studies have shown promising therapeutic effects of PRP; however there are still many controversies regarding its composition, properties and clinical efficacy. The aim of this study was to evaluate the influence of different platelet concentrations on the rheological properties and growth factor (GF) release profile of PRP-gels. In addition, the viability of incorporated bone marrow-derived human mesenchymal stem cells (MSCs) was investigated.

PRP (containing $1000x10^3$, $2000x10^3$, $10000x10^3$ platelets/µl) was prepared from human platelet concentrates. Platelet activation and gelification was achieved by addition of human thrombin. Viscoelastic properties of PRP-gels were evaluated by rheological studies. The release of GFs and inflammatory proteins was measured using a membrane based protein array and ELISA. MSC viability and proliferation in PRP-gels was assessed over 7 days by cell viability staining. Cell proliferation was examined using DNA quantification.

Regardless of the platelet content, all tested PRP-gels showed effective crosslinking. A positive correlation between protein release and the platelet concentration was observed at all time points. Among the detected proteins the chemokine CCL5 was the most abundant. The greatest release appeared within the first 4 hours after gelification. MSCs could be successfully cultured in PRP-gels over 7 days with the highest cell viability and DNA content found in PRP-gels with $1000x10^3$ platelets/µl.

The results of this study suggest that PRP-gels represent a suitable carrier for both cell- and growth factor delivery for tissue engineering. Notably, a platelet concentration of $1000x10^3$ platelets/µl appeared to provide the most favorable environment for MSCs. Thus, the platelet concentration is an important consideration for the clinical application of PRP-gels.

INTRODUCTION

Platelets are known to play an important role in hemostasis and in the process of tissue healing. Upon activation they release numerous growth factors (GFs) and cytokines such as transforming growth factor β-1 (TGFβ-1), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), insulin-like growth factor 1 (IGF-I), basic fibroblast growth factor (bFGF), endothelial growth factor (EGF) and hepatocyte growth factor (HGF) (1). Platelet released GFs are not only known to enhance recruitment, proliferation and differentiation of cells (2-4), but they are also thought to play a role in angiogenesis (5-7) and inflammation (8, 9). As such, platelet rich plasma (PRP) has gained popularity in tissue repair and regeneration and serves as a treatment option for various medical conditions in human and veterinary medicine (10-13). Despite many reports discussing the clinical application of PRP, there are still controversies regarding its preparation, delivery method, and clinical efficacy. Many protocols and commercially available systems have been used for the production of PRP, although each of these methods generates a different final product, where the concentration of platelets, leukocytes and the amount of released protein varies (14, 15) often leading to conflicting results (16, 17). The optimal amount of platelets in PRP is still unknown and it is not clear whether its specific formulation correlates with levels of released growth factors (18, 19). Many proteins released from platelets possess inflammation-modulating properties; hence the potential benefit they may exert at the injury site is likely to be tissue dependent.

PRP can be produced using whole blood centrifugation or apheresis. Several commercial centrifugation systems are available and offer a simple, minimal manipulative preparation of autologous PRP, however the platelet counts and enrichment levels are variable (ranging from physiological platelet concentrations up to 18-fold enrichment) and only few systems allow adjustments by the user (according to manufacturer`s brochures and (20)). Platelet concentrates from whole blood centrifugation contain erythrocytes and leukocytes. In contrast, apheresis allows the exclusion of components, which are undesired, since the accumulation of leukocytes might be deleterious for the healing process (21, 22).

The form in which PRP is used depends on the application and target tissue. Many clinicians use PRP in an inactivated liquid form that can be easily injected. After application, platelets are activated by contact with the surrounding tissue collagen (23). Alternatively, an exogenous activator like chitosan, batroxobin, thrombin, calcium chloride ($CaCl_2$) or a combination of the latter two can be used to form a PRP-gel (24-28). PRP-gels have been used as an autologous hydrogel containing bioactive molecules, locally enhancing tissue healing (29-34). The composition of PRP-gels in these studies was however highly variable, mainly due to differences in PRP preparation and activation methods, which in turn leads to significant differences in platelet concentration, leukocyte content and growth factor release (20). Previous studies have reported a successful culture of cells incorporated into PRP-gels suggesting that the gel environment enhances viability and proliferation of encapsulated cells (33, 35). Kawasumi et al. encapsulated rat bone marrow mesenchymal stem cells (MSCs) in PRP-gels containing different platelet concentration, ranging from 0.05 to $4.4x10^6$ platelets/µl. The authors demonstrated a platelet-concentration dependent stimulation of cell proliferation in vitro as well as enhanced bone formation in a rat osteotomy in vivo (35).

Although previously applied for various applications in vivo, we feel that PRP-gels have not sufficiently been characterized. PRP may be homemade or obtained using clinical systems, all these protocols/systems result in different platelet and leukocyte concentrations, with additional variability induced by donor variations (36). Here we investigated three different platelet concentrations in our PRP-gels (1000, 2000, 10000 x 10^3 platelets/µl), referring to a 5x, 10x or 50x platelet enrichment assuming an average concentration of 200 x 10^3 platelets/µl in healthy human donors. Most studies using PRP-gels, particularly in the context of bone repair, have used similar concentrations (e.g. 48 – 4358 x 10^3 platelets/µl (35) and 1040 x 10^3 platelets/µl (34)), while other studies do not indicate the platelet concentrations in their PRP preparations. The aim of this study was to systematically characterise different compositions of PRP-gels created from platelet concentrates and to compare these gels with respect to their rheological properties, the content- and release profile of GFs and finally to assess the viability of incorporated mesenchymal stem cells (MSCs).

MATERIALS AND METHODS

Preparation of PRP and PRP-gels

PRP was produced from human leukocyte-depleted platelet concentrates (blood bank, Kantonspital Graubünden, Chur, Switzerland) obtained by apheresis and collected into transfusion bags containing acid citrate dextrose (ACD-A) with a thrombocyte concentration of $2.4x10^{11}$ per 240 ml of plasma ($1000x10^3$ thrombocytes/µl) and containing less than $5x10^5$ leukocytes. Based on the assumption that the average platelet count in healthy human adults is $200x10^3$/µl, this platelet concentrate was considered to be 5 times enriched. In order to produce PRP with higher platelet concentrations, the platelet concentrates were centrifuged at 2000 g for 20 min and the resulting pellet resuspended in the appropriate volume of platelet-depleted plasma. PRP was sonicated for 15 min and stored at -20°C until use. If not stated otherwise PRP-gels were produced by pipetting 3 µl human thrombin (final concentration 5 U/ml, Tisseel, Baxter) into a well of a 48-well cell culture plate (Falcon). The plate was placed on an orbital shaker and 312 µl of PRP slowly added. Gels were incubated at room temperature for 15 min for gelification.

Rheology

The viscoelastic properties of PRP-gels were investigated via oscillatory rheology. Discs of PRP-gels of 25 mm diameter and 0.2 mm thickness were prepared with a dedicated mold. Thrombin concentration was 5 U/ml, 8 U/ml and 16 U/ml. All rheological measurements were performed using an Anton Paar MCR-302 rheometer equipped with a 25 mm parallel plate, a Peltier temperature control system and an insulating thermostatic hood. First, the samples were screened with an amplitude sweep at 10 rad/sec and 20°C. This measurement allows the individuation of a suitable deformation range, which should be within the linear viscoelastic region. A strain of 1% was verified to be within this limit for all tested gels and further used for each sample. A frequency sweep (measure of viscoelastic shear moduli as a function of the frequency of deformation) between 0.1 and 25 rad/sec at 20°C was finally performed. For each specimen, 3 independent freshly-prepared samples were measured, and the results reported as mean ± standard error of the mean..

GFs-release from PRP-gels

GF release was measured from PRP-gels incubated in 800 µl of phosphate buffer saline (PBS) at 37°C at different time points after gelification (30 min – 168 h). At every time point, 400 µl of conditioned PBS was collected for analysis and removed volume was replaced with new 400 µl of PBS. ELISA assays were performed to quantify CCL5/RANTES (DuoSet®, R&D Systems), TGFβ-1, VEGF, PDGF-AB and PDGF-BB (all Quantikine®, R&D Systems) according to the manufacturer´s instructions. Samples were diluted according to the platelet concentration of the gels. Absorbance was measured at 450 nm and at 560 nm in order to eliminate background signal using a Victor3™ plate reader (Perkin Elmer).

The presence of inflammatory proteins in conditioned PBS from different PRP-gels (pooled samples from two PRP donors, collected 12h after gelification) was investigated using a human inflammation protein array (RayBio®, C-series AAH-INF-3-4) according to the manufacturer`s instructions. Briefly, the membranes were incubated with 1 ml PRP supernatant overnight at 4°C on an orbital shaker. Chemiluminescence (20 min exposure time) was detected using the ChemiGenius Bio Imaging System (Syngene, UK). Semi-quantification was performed by averaging values from duplicated spots and measuring signal intensity using ImageJ (Rasband, NIH). Data are presented as relative abundance following normalization to the RayBio® intrinsic positive control.

MSC isolation, culture and encapsulation in PRP-gels

Bone marrow (BM) aspirates were obtained from the iliac crest or vertebral body of patients undergoing elective orthopaedic surgery (mean age: 56.5 years, range: 26-89 years; 1 male and 8 female). Informed consent was given by all patients and approval by local ethical authorities obtained (KEK Bern 126/03). Mononucleated cells (MNCs) were isolated from BM aspirates by density centrifugation with Ficoll™ (Histopaque-1077, Sigma). MNCs were seeded tissue culture flasks at a density of $5x10^4$ cells per cm^2 in αMEM (Gibco) containing 10% FBS (Pan), 5 ng/ml bFGF (R&D Systems). After 4 days in culture, non-adherent haematopoietic cells were removed. Mesenchymal stem cells (MSCs) selected by adherence to cell culture plastic were further expanded with a change of medium every 3 days. Cells were passaged when 80% confluency was reached. All experiments were

performed with cells at the second passage. MSCs used as monolayer controls were seeded at the density of $3x10^3$ cells/cm^2 in 6-well tissue culture plates.

For the incorporation of MSCs into PRP-gels, PRP aliquots were thawed and in order to avoid donor variability, 3 PRP donors with the same blood group pooled. MSCs were seeded in PRP-gels at a density $1.25x10^5$ cells per gel in a total volume of 315 µl. Gelification was performed by using a final concentration of 5 U/ml thrombin as described above. Gels were incubated at 37°C in αMEM containing 10% FBS, 5 ng/ml basic-FGF, 100 U/ml PenStrep and 5 µM ε-aminocaproic acid (Sigma).

Viability of MSCs

The viability of MSCs encapsulated in PRP-gels was determined at day 0 (3 hours after seeding), day 3 and day 7. PRP-gels were stained with a solution of 100 pg/ml calcein AM and 1 pg/ml ethidium homodimer-1 (both Sigma) in 1 ml of αMEM for 30 minutes at 37°C and visualized with an inverted phase contrast microscope (Olympus CK40) equipped with a fluorescence lamp.

For semi-quantitative analysis of cell viability following recovery from PRP-gels, 1 h, 3 and 7 days after cell encapsulation PRP gels underwent enzymatic digestion in type II collagenase (600 U/ml; Worthington). The number of retrieved viable cells was determined by trypan blue (Sigma) exclusion. Cell viability was further determined by propidium iodide (PI 4 µg/ml; Sigma) staining and flow cytometry (BD FACS Aria III). To define the Forward Scatter (FSC) and Side Scatter (SSC) region for the analysis a PRP-gel without cells was used to exclude platelet debris.

DNA content

At day 0 (5 hours after seeding), day 3 and day 7, PRP-gels were subjected to digestion in proteinase K (0.5 mg/ml,Roche) for 16 h at 56°C. The DNA content was measured using an ultrasensitive fluorescent nucleic acid stain PicoGreen® (Invitrogen) which allows quantitating double-stranded DNA at concentrations as low as 25 pg/ml. A calf thymus DNA standard (Invitrogen) was used to create a five-point standard curve from 2 ng/ml to 2 µg/ml.Measurements were performed using a Victor3™ (Perkin Elmer) plate reader at 485 nm excitation and 535 nm emission wavelength.

Statistical analysis

All values are shown as mean±standard error of the mean (SEM). Statistical analysis was performed with the Kruskal-Wallis test using GraphPad Prism. $p<0.05$ was considered as statistically significant.

RESULTS

Formation of PRP-gels

All gel formulations could be cultured for 7 days without evident visual changes in shape or size (Fig.1). Rheological measurement revealed that for all tested gels the G' was over G" demonstrating an effective cross-linking of samples. Neither the platelet concentration (Fig.2A), nor the concentration of thrombin (Fig.2B) significantly affected the viscoelastic properties of the gels. However, we found that PRP gels with $2000x10^3$ platelets/µl were slightly stiffer than PRP-gels with $10000x10^3$ or with $1000x10^3$ platelets/µl (Fig.2A), with the lowest values observed for the lowest platelet concentration. When testing the influence of different thrombin concentrations on PRP-gels with the same platelet content we found that the lowest final thrombin concentration (5 U/ml) showed the highest values of storage and loss moduli and was therefore chosen for further experiments (Fig.2B).

Growth Factors release from PPR-gels

First the release of GFs that are known to be important signaling molecules in tissue repair were examined (1). TGFβ-1, PDGF-AB, PDGF-BB, VEGF could be detected in PBS conditioned by PRP from all tested PRP-gels from all platelet concentrations (Fig.3). Additionally, the cumulative and temporal release of GFs from PRP-gels was investigated (Fig.3.). A positive correlation between protein secretion and platelet concentration was observed at all time points and platelet concentrations. Overall, most of the release of GFs appeared within the first 4 hours after gelification. A comparatively lower sustained release of GFs was observed at later time points (Fig.3). 12 hours after gelification the cumulative release of activated TGFβ-1 (Fig.3 A right) showed the highest values in PRP gels with $10000x10^3$ platelets/µl reaching 13.08±3.48 ng, followed by $2000x10^3$ platelets/µl PRP-gels with 4.07±0.61 ng and $1000x10^3$ platelets/µl PRP with 2.62±0.26 ng. When compared to TGFβ-1 the values of cumulative release of PDGF-AB (Fig.3B) were lower for the highest (9.34±1.9 ng) and middle (3.54±0.84 ng) platelet concentration but higher (3.81±0.29 ng) for PRP-gels with $10000x10^3$ platelets/µl. Release of PDGF-BB at 12 h was clearly lower for all PRP-gels reaching 0.23±0.03 ng,

0.34±0.04 ng and 1.05±0.25 ng in 1000x10^3, 2000x10^3 and 10000x10^3 platelets/µl gels respectively. VEGF release was the lowest when compared to the other GFs.

PRP-gels release inflammatory proteins

Due to existing controversies regarding possible pro-inflammatory effects of PRP, a screening array was performed in order to identify the most abundant inflammatory proteins released from PRP-gels. A total of 28 proteins known to play a role in inflammation was detected (Fig.4). The chemokine CCL5 was released in the highest amounts compared to other factors, followed by PDGF-BB, interferon-gamma induced protein 10 (IP10), interleukin-6 soluble receptor (IL-6sR), macrophage inflammatory protein 1 beta (MIP-1β), metallopeptidase inhibitor 2 (TIMP2) and intracellular adhesion molecule (ICAM) (Fig.4A). Other proteins such as tumor necrosis factor-beta (TNFβ), interferon gamma (INF-γ), chemokine I-309 (I-309) and eotaxin-2 were found at comparably lower levels while some factors including TNFα, interleukin 2 (IL-2), IL-3, IL-5 and IL-16 were detected only in supernatants from PRP-gels with 10000x10^3 platelets/µl. Overall, the relative abundance of inflammatory proteins positively correlated with increasing platelet concentration in PRP-gels. Based on these findings, the release of CCL5 over time was investigated by ELISA (Fig.4B and C). The total amount of secreted CCL5 was significantly higher, when compared to TGFβ-1, PDGF and VEGF, also measured by ELISA. After 12h post gelification the cumulative release of CCL5 (Fig.4C) showed the highest values in 10000x103 platelets/µl gels (211.2±97.5ng) followed by 2000x10^3 platelets/µl gels (77.1±6.8 ng) with lowest values in 1000x10^3 platelets/µl gels (37.3±3.1 ng). The peak of CCL5-release appeared within the first 2 hours after gelification and was sustained at moderate levels over the next 5 days (Fig.4B).

Platelet concentration in PRP-gels influences MSC behavior

MSCs could be successfully cultured for 7 days in all PRP-gels, regardless of the platelet concentration. A difference in DNA content between different platelet concentrations was observed directly after cell encapsulation (Fig. 5A). The highest DNA content was found in 1000x10^3

platelets/µl gels, followed by $2000x10^3$ and 10000 platelets/µl gels. The same trend was noticed at day 3 and day 7. Overall, the DNA content at day 7 in all tested PRP-gels was approximately 2-fold higher when compared to day 0.

PI-staining revealed an average percentage of viable cells at day 0 (three hours after seeding) of 98.3±0.14%, 93.3±2.43% and 93.2±1.70% in $1000x10^3$, $2000x10^3$ and $10000x10^3$ platelets/µl gels, respectively (Fig.5B). At day 3 the highest cell viability was detected for PRP-gels with $1000x10^3$ platelets/µl (96.8±0.07%) and significant different ($p<0.05$) compared to 89.8±2.14% in $10000x10^3$ platelets/µl gels. At day 7 the cell viability in gels containing the lowest concentration of platelets was the highest, reaching 92.4±0.88%. For PRP-gels with $2000x10^3$ and 10000 $x10^3$ platelets/µl the viability was 86.73±2.46% and 87.13±2.75%, respectively. Live/dead staining showed a change in the cell morphology over time, with rounded cells on day 0 to a spindel-shaped morphology at day 3 and day 7. The density of cells seeded in gels increased over time (Fig.5C).

DISCUSSION

An *in vitro* characterization of PRP-gels created from platelet concentrates, activated by human thrombin and composed of different platelet concentrations was performed. It could be shown that the platelet concentration within PRP is a crucial factor determining gel properties and influencing behavior of encapsulated cells.

To the best of our knowledge, no study has investigated the viscoelastic properties of PRP-gels. For the PRP-gels prepared in the present study a prevalence of storage over loss modulus was observed for every platelet and thrombin concentration. This is a direct consequence of the crosslinking at the molecular level. Macroscopically, this means that the material tends to maintain its shape rather than creeping and spreading like a viscous fluid. This property, in combination with a composition which is more than 95% water, allowing nutrition and waste transport, makes hydrogels effective cell carriers. Here, it was shown that the highest storage modulus was achieved by PRP-gels with $1000x10^3$ and $2000x10^3$ platelets/µl gelified with 5 U/ml of thrombin. Higher platelet content in PRP-gels did not result in increased stiffness. A possible explanation for this is that the proper balance between concentration of platelets and thrombin has to be reached in order to effectively gelify PRP, otherwise some of the platelets stay in inactivated form and do not contribute to gel formation. Furthermore, a dense matrix of PRP-gels containing highly concentrated platelets may prevent a proper diffusion of thrombin within the gel. Additionally, platelets at a higher number may act as external bodies hindering the contact between the fibrinogen strands and its effective crosslinking. Despite the efficient crosslinking PRP-gels do not have a stiffness of a load-bearing structure; hence their mechanical properties are very limited. However, previous reports indicate that stiffness of hydrogels may influence behavior and differentiation potential of incorporated MSCs (37, 38).

The importance of the platelet activator is not only limited to induction of platelet aggregation; an exogenous triggering factor is also necessary, to ensure a complete release of GFs (39). In the current study thrombin was used and it was hypothesized that this will result in a rapid gel formation,

degranulation of platelets and an immediate release of GFs. It was demonstrated that the majority of GFs was indeed released within the first 6h after the gel has formed. The results of this study agree with previous reports indicating that most of the release occurs between 1-6 hours after clot formation (40, 41). Interestingly, even 12h after gelification GF-release could be detected, however at clearly lower concentrations. One possible explanation for this is that some of the GFs remain captured in the PRP-gel and as a consequence are secreted at later time points. It is likely that GFs located peripherally in the gel may diffuse faster than centrally situated ones. Furthermore, some GFs may be initially captured within fibrin mesh of PRP-gel; hence their release occurs at later time points. Also, the here presented in vitro investigation does not completely reflect the in vivo situation where tissue specific factors, proteins and body fluids would interact with the applied material. In addition, various cells migrating into the PRP-gel may further influence the process of gel degradation and GF release. Rademakers and colleagues studied PRP degradation (platelet concentration ~1000 x 10^3 platelets/µl) in an animal study and found that the weight of autologous platelet clots was reduced to 16% of the initial weight after 30 min of incubation in the pericardial sac (42). *In vivo,* tissue-related factors regulate the secretion of GFs from platelets. In addition, recruited or co-transplanted cells may also secrete GFs (43). Another question is, whether the released amount of GFs is physiologically relevant. Further studies are necessary to investigate the optimal concentration of GFs at the injury site and whether lower amounts still have a positive effect on tissue repair. Interestingly, a study investigating the mRNA profile of human platelets suggests that platelets may synthesize some cytokines and GF *de novo* (44).

The results of this study support the hypothesis that release of proteins from PRP-gels is correlated with the platelet concentration. Similar results have been reported previously (39), however also opposite findings, indicating a poor correlation between platelet number and GF content have been reported (36, 45). Among the screened GFs in supernatants from our PRP-gels, CCL5 was released in the greatest amounts followed by TGF-β1. TGF-β has been proposed for the treatment of chronic inflammatory diseases, soft and hard tissue regeneration and autoimmune diseases (46). It has been shown that TGF-β signaling enhances maturation of osteoblasts, and bone matrix deposition (47).

Accordingly, several studies suggest positive effects of TGF-β when used for bone repair (48-51). PDGF-AB and PDGF-BB were other GFs found in supernatants from PRP-gels in this study. PDGFs are known to promote the proliferation of bone cells and influences osteoblastic mitogenesis (52). Based on that, PRP-gel may serve as a local delivery system of TGF-β and PDGF into bone defects. Considering the pro-angiogenic effects of PDGF and VEGF (6) PRP-gels may further enhance bone healing by supporting re-vascularization.

In supernatants from PRP-gels many chemokines and mediators that are known to possess pro-inflammatory properties could be detected in this study. CCL5 was released at highest levels, peaking at 1 hour after platelet activation and still detectable up to 5 days after. Interestingly, in a study investigating the release of GFs from equine platelets it was found, that secretion of CCL5 was not associated with platelet activation and persisted longer than other GFs suggesting that CCL5-release may be regulated differently (41). CCL5 may have deleterious effects on the healing process, since it attracts activated leukocytes to the injury site and their accumulation may abolish beneficial effects of GFs (53, 54). Increased infiltration of leukocytes may lead to tissue damages and a persistent inflammatory phase (55, 56). On the other hand, an early inflammatory phase is an essential component initiating the healing process, even though many mediators can either stimulate or suppress tissue repair during inflammation (57). Furthermore, it has been shown that CCL5 is a main chemoattractant released by degenerated discs and its presence may enhance the migration of MSCs and progenitor cells towards injury sites (58). Another study investigating the role of CCL5 in mouse wound healing suggests that this protein induces migration of endothelial progenitor cells (EPCs) (59). These findings are particularly interesting for tissue engineering strategies, because application of PRP-gels that locally release CCL5 may intensify cell mobilization, enhance vascularization and therefore improve the healing process.

In this study MSCs could be successfully cultured in all tested PRP-gel compositions. The rationale behind encapsulating MSCs into gels was to investigate whether PRP-gels could be used as a scaffold and delivery system of growth factors and cells at the injury site. From the clinical point of view it could be potentially beneficial in patients suffering from impaired wound healing and tissue loss such

as bony defects or cartilage lesions, when growth factors and cells are needed in order to enhance repair. We found that cell survival and proliferation was greatest in gels containing the lowest platelet concentration ($1000x10^3$ platelets/µl). A study investigating the proliferation of MSCs and adipose tissue derived stem cells encapsulated into PRP-gels has shown a 3- to 5-fold increase of the cell number after 7 days of culture (33). In that study the platelet number in PRP-gels was 6-fold higher than the average concentration in whole blood. Similar findings were reported in a study investigating the effect of platelet concentration on bone healing, suggesting that a moderate platelet concentration (2-6-fold concentrated) gives optimal results (18). These studies along with our results suggest that a lower platelet content in PRP seems to be more favorable for incorporated cells. Thus most of the commercially available systems based on whole blood centrifugation usually yield to a 2x to 3x platelet enrichment levels compared to baseline when used in standard settings (25). Therefore, our preparation method of PRP-gels allows obtaining optimal platelet concentration in a clinically feasible manner.

In summary, the results of this study demonstrate that PRP-gels are stable hydrogels, which can effectively deliver bioactive substances and MSCs for various clinical applications. Importantly, the platelet concentration seems to determine the efficacy and properties of PRP-gels. It was shown that a concentration of $1000x10^3$ platelets/µl in PRP provides an optimal environment for encapsulated MSCs. With regards to clinical application, the concentration and balance of pro- and anti-inflammatory substances should be further investigated in order to maximize efficacy.

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DISCLOSURE STATEMENT

The authors have no conflicts of interest.

REFERENCES

1. Harrison, P., and Cramer, E.M. Platelet alpha-granules. Blood reviews **7**, 52, 1993.

2. Anitua, E., Andia, I., Sanchez, M., Azofra, J., del Mar Zalduendo, M., de la Fuente, M., Nurden, P., and Nurden, A.T. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. Journal of orthopaedic research : official publication of the Orthopaedic Research Society **23**, 281, 2005.

3. Verrier, S., Meury, T.R., Kupcsik, L., Heini, P., Stoll, T., and Alini, M. Platelet-released supernatant induces osteoblastic differentiation of human mesenchymal stem cells: potential role of BMP-2. European cells & materials **20**, 403, 2010.

4. Kruger, J.P., Hondke, S., Endres, M., Pruss, A., Siclari, A., and Kaps, C. Human platelet-rich plasma stimulates migration and chondrogenic differentiation of human subchondral progenitor cells. Journal of orthopaedic research : official publication of the Orthopaedic Research Society **30**, 845, 2012.

5. Herrmann, M., Binder, A., Menzel, U., Zeiter, S., Alini, M., and Verrier, S. CD34/CD133 enriched bone marrow progenitor cells promote neovascularization of tissue engineered constructs in vivo. Stem cell research **13**, 465, 2014.

6. Italiano, J.E., Jr., Richardson, J.L., Patel-Hett, S., Battinelli, E., Zaslavsky, A., Short, S., Ryeom, S., Folkman, J., and Klement, G.L. Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. Blood **111**, 1227, 2008.

7. Lippross, S., Loibl, M., Hoppe, S., Meury, T., Benneker, L., Alini, M., and Verrier, S. Platelet released growth factors boost expansion of bone marrow derived CD34(+) and CD133(+) endothelial progenitor cells for autologous grafting. Platelets **22**, 422, 2011.

8. Che, W., Lerner-Marmarosh, N., Huang, Q., Osawa, M., Ohta, S., Yoshizumi, M., Glassman, M., Lee, J.D., Yan, C., Berk, B.C., and Abe, J. Insulin-like growth factor-1 enhances inflammatory responses in endothelial cells: role of Gab1 and MEKK3 in TNF-alpha-induced c-Jun and NF-kappaB activation and adhesion molecule expression. Circulation research **90**, 1222, 2002.

9. Andrae, J., Gallini, R., and Betsholtz, C. Role of platelet-derived growth factors in physiology and medicine. Genes & development **22**, 1276, 2008.

10. Liddle, A.D., and Rodriguez-Merchan, E.C. Platelet-Rich Plasma in the Treatment of Patellar Tendinopathy: A Systematic Review. The American journal of sports medicine 2014.

11. Paques, M., Chastang, C., Mathis, A., Sahel, J., Massin, P., Dosquet, C., Korobelnik, J.F., Le Gargasson, J.F., and Gaudric, A. Effect of autologous platelet concentrate in surgery for idiopathic macular hole: results of a multicenter, double-masked, randomized trial. Platelets in Macular Hole Surgery Group. Ophthalmology **106**, 932, 1999.

12. Zheng, C., Zhu, Q., Liu, X., Huang, X., He, C., Jiang, L., and Quan, D. Improved peripheral nerve regeneration using acellular nerve allografts loaded with platelet-rich plasma. Tissue engineering Part A **20**, 3228, 2014.

13. Waselau, M., Sutter, W.W., Genovese, R.L., and Bertone, A.L. Intralesional injection of platelet-rich plasma followed by controlled exercise for treatment of midbody suspensory ligament desmitis in Standardbred racehorses. Journal of the American Veterinary Medical Association **232**, 1515, 2008.

14. Magalon, J., Bausset, O., Serratrice, N., Giraudo, L., Aboudou, H., Veran, J., Magalon, G., Dignat-Georges, F., and Sabatier, F. Characterization and comparison of 5 platelet-rich plasma preparations in a single-donor model. Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association **30**, 629, 2014.

15. Mazzocca, A.D., McCarthy, M.B., Chowaniec, D.M., Cote, M.P., Romeo, A.A., Bradley, J.P., Arciero, R.A., and Beitzel, K. Platelet-rich plasma differs according to preparation method and human variability. The Journal of bone and joint surgery American volume **94**, 308, 2012.

16. Sheth, U., Simunovic, N., Klein, G., Fu, F., Einhorn, T.A., Schemitsch, E., Ayeni, O.R., and Bhandari, M. Efficacy of autologous platelet-rich plasma use for orthopaedic indications: a meta-analysis. The Journal of bone and joint surgery American volume **94**, 298, 2012.

17. de Vos, R.J., Windt, J., and Weir, A. Strong evidence against platelet-rich plasma injections for chronic lateral epicondylar tendinopathy: a systematic review. British journal of sports medicine **48**, 952, 2014.

18. Weibrich, G., Hansen, T., Kleis, W., Buch, R., and Hitzler, W.E. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone **34**, 665, 2004.

19. Giusti, I., D'Ascenzo, S., Manco, A., Di Stefano, G., Di Francesco, M., Rughetti, A., Dal Mas, A., Properzi, G., Calvisi, V., and Dolo, V. Platelet concentration in platelet-rich plasma affects tenocyte behavior in vitro. BioMed research international **2014**, 630870, 2014.

20. Mazzucco, L., Balbo, V., Cattana, E., Guaschino, R., and Borzini, P. Not every PRP-gel is born equal. Evaluation of growth factor availability for tissues through four PRP-gel preparations: Fibrinet, RegenPRP-Kit, Plateltex and one manual procedure. Vox sanguinis **97**, 110, 2009.

21. Martin, P., and Leibovich, S.J. Inflammatory cells during wound repair: the good, the bad and the ugly. Trends in cell biology **15**, 599, 2005.

22. Toumi, H., and Best, T.M. The inflammatory response: friend or enemy for muscle injury? British journal of sports medicine **37**, 284, 2003.

23. Roberts, D.E., McNicol, A., and Bose, R. Mechanism of collagen activation in human platelets. The Journal of biological chemistry **279**, 19421, 2004.

24. Shen, E.C., Chou, T.C., Gau, C.H., Tu, H.P., Chen, Y.T., and Fu, E. Releasing growth factors from activated human platelets after chitosan stimulation: a possible bio-material for platelet-rich plasma preparation. Clinical oral implants research **17**, 572, 2006.

25. DeLong, J.M., Russell, R.P., and Mazzocca, A.D. Platelet-rich plasma: the PAW classification system. Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association **28**, 998, 2012.

26. Betoni-Junior, W., Dechichi, P., Esteves, J.C., Zanetta-Barbosa, D., and Magalhaes, A.E. Evaluation of the bone healing process utilizing platelet-rich plasma activated by thrombin and calcium chloride: a histologic study in rabbit calvaria. The Journal of oral implantology **39**, 14, 2013.

27. Cheng, C.M., Meyer-Massetti, C., and Kayser, S.R. A review of three stand-alone topical thrombins for surgical hemostasis. Clinical therapeutics **31**, 32, 2009.

28. Mazzucco, L., Balbo, V., Cattana, E., and Borzini, P. Platelet-rich plasma and platelet gel preparation using Plateltex. Vox sanguinis **94**, 202, 2008.

29. Carter, C.A., Jolly, D.G., Worden, C.E., Sr., Hendren, D.G., and Kane, C.J. Platelet-rich plasma gel promotes differentiation and regeneration during equine wound healing. Exp Mol Pathol **74**, 244, 2003.

30. Rai, A.K., and Sharma, N.K. Phosphate metabolism in the cyanobacterium Anabaena doliolum under salt stress. Curr Microbiol **52**, 6, 2006.

31. Driver, V.R., Hanft, J., Fylling, C.P., Beriou, J.M., and Autologel Diabetic Foot Ulcer Study, G. A prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers. Ostomy Wound Manage **52**, 68, 2006.

32. Sun, Y., Feng, Y., Zhang, C.Q., Chen, S.B., and Cheng, X.G. The regenerative effect of platelet-rich plasma on healing in large osteochondral defects. Int Orthop **34**, 589, 2010.

33. Xie, X., Wang, Y., Zhao, C., Guo, S., Liu, S., Jia, W., Tuan, R.S., and Zhang, C. Comparative evaluation of MSCs from bone marrow and adipose tissue seeded in PRP-derived scaffold for cartilage regeneration. Biomaterials **33**, 7008, 2012.

34. Malhotra, A., Pelletier, M., Oliver, R., Christou, C., and Walsh, W.R. Platelet-rich plasma and bone defect healing. Tissue engineering Part A **20**, 2614, 2014.

35. Kawasumi, M., Kitoh, H., Siwicka, K.A., and Ishiguro, N. The effect of the platelet concentration in platelet-rich plasma gel on the regeneration of bone. The Journal of bone and joint surgery British volume **90**, 966, 2008.

36. Weibrich, G., Kleis, W.K., Kunz-Kostomanolakis, M., Loos, A.H., and Wagner, W. Correlation of platelet concentration in platelet-rich plasma to the extraction method, age, sex, and platelet count of the donor. Int J Oral Maxillofac Implants **16**, 693, 2001.

37. Brandl, F., Sommer, F., and Goepferich, A. Rational design of hydrogels for tissue engineering: impact of physical factors on cell behavior. Biomaterials **28**, 134, 2007.

38. Duarte Campos, D.F., Blaeser, A., Korsten, A., Neuss, S., Jakel, J., Vogt, M., and Fischer, H. The stiffness and structure of three-dimensional printed hydrogels direct the differentiation of mesenchymal stromal cells toward adipogenic and osteogenic lineages. Tissue engineering Part A **21**, 740, 2015.

39. McCarrel, T., and Fortier, L. Temporal growth factor release from platelet-rich plasma, trehalose lyophilized platelets, and bone marrow aspirate and their effect on tendon and ligament gene expression. Journal of orthopaedic research : official publication of the Orthopaedic Research Society **27**, 1033, 2009.

40. Su, C.Y., Kuo, Y.P., Nieh, H.L., Tseng, Y.H., and Burnouf, T. Quantitative assessment of the kinetics of growth factors release from platelet gel. Transfusion **48**, 2414, 2008.

41. Dunkel, B., Bolt, D.M., Smith, R.K., and Cunningham, F.M. Stimulus-dependent release of tissue-regenerating factors by equine platelets. Equine veterinary journal **44**, 346, 2012.

42. Rademakers, L.M., Grundeman, P.F., Bolderman, R.W., van der Veen, F.H., and Maessen, J.G. Stability of an autologous platelet clot in the pericardial sac: An experimental and clinical study. J Thorac Cardiovasc Surg **137**, 1190, 2009.

43. Hoch, A.I., Binder, B.Y., Genetos, D.C., and Leach, J.K. Differentiation-dependent secretion of proangiogenic factors by mesenchymal stem cells. PloS one **7**, e35579, 2012.

44. Bugert, P., Dugrillon, A., Gunaydin, A., Eichler, H., and Kluter, H. Messenger RNA profiling of human platelets by microarray hybridization. Thrombosis and haemostasis **90**, 738, 2003.

45. Eppley, B.L., Woodell, J.E., and Higgins, J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. Plastic and reconstructive surgery **114**, 1502, 2004.

46. Roberts, A.B., and Sporn, M.B. Physiological actions and clinical applications of transforming growth factor-beta (TGF-beta). Growth factors **8**, 1, 1993.

47. Shen, J., Li, S., and Chen, D. TGF-beta signaling and the development of osteoarthritis. Bone research **2**2014.

48. Shakir, S., MacIsaac, Z.M., Naran, S., Smith, D.M., Bykowski, M.R., Cray, J.J., Craft, T.K., Wang, D., Weiss, L., Campbell, P.G., Mooney, M.P., Losee, J.E., and Cooper, G.M. Transforming growth factor Beta 1 augments calvarial defect healing and promotes suture regeneration. Tissue engineering Part A **21**, 939, 2015.

49. Farea, M., Husein, A., Halim, A.S., Abdullah, N.A., Mokhtar, K.I., Lim, C.K., Berahim, Z., and Mokhtar, K. Synergistic effects of chitosan scaffold and TGFbeta1 on the proliferation and osteogenic differentiation of dental pulp stem cells derived from human exfoliated deciduous teeth. Archives of oral biology **59**, 1400, 2014.

50. Fu, H., Rahaman, M.N., Brown, R.F., and Day, D.E. Evaluation of bone regeneration in implants composed of hollow HA microspheres loaded with transforming growth factor beta1 in a rat calvarial defect model. Acta biomaterialia **9**, 5718, 2013.

51. Beck, L.S., Amento, E.P., Xu, Y., Deguzman, L., Lee, W.P., Nguyen, T., and Gillett, N.A. TGF-beta 1 induces bone closure of skull defects: temporal dynamics of bone formation in defects exposed to rhTGF-beta 1. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research **8**, 753, 1993.

52. Everts, P.A., Knape, J.T., Weibrich, G., Schonberger, J.P., Hoffmann, J., Overdevest, E.P., Box, H.A., and van Zundert, A. Platelet-rich plasma and platelet gel: a review. The Journal of extra-corporeal technology **38**, 174, 2006.

53. Schall, T.J., Bacon, K., Toy, K.J., and Goeddel, D.V. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. Nature **347**, 669, 1990.

54. Rot, A., Krieger, M., Brunner, T., Bischoff, S.C., Schall, T.J., and Dahinden, C.A. RANTES and macrophage inflammatory protein 1 alpha induce the migration and activation of normal human eosinophil granulocytes. The Journal of experimental medicine **176**, 1489, 1992.

55. von Hundelshausen, P., and Schmitt, M.M. Platelets and their chemokines in atherosclerosis-clinical applications. Frontiers in physiology **5**, 294, 2014.

56. Gleissner, C.A., von Hundelshausen, P., and Ley, K. Platelet chemokines in vascular disease. Arteriosclerosis, thrombosis, and vascular biology **28**, 1920, 2008.

57. Pape, H.C., Marcucio, R., Humphrey, C., Colnot, C., Knobe, M., and Harvey, E.J. Trauma-induced inflammation and fracture healing. Journal of orthopaedic trauma **24**, 522, 2010.

58. Pattappa, G., Peroglio, M., Sakai, D., Mochida, J., Benneker, L.M., Alini, M., and Grad, S. CCL5/RANTES is a key chemoattractant released by degenerative intervertebral discs in organ culture. European cells & materials **27**, 124, 2014.

59. Ishida, Y., Kimura, A., Kuninaka, Y., Inui, M., Matsushima, K., Mukaida, N., and Kondo, T. Pivotal role of the CCL5/CCR5 interaction for recruitment of endothelial progenitor cells in mouse wound healing. The Journal of clinical investigation **122**, 711, 2012.

FIGURE LEGENDS

Figure 1. PRP with 2000x10^3 platelets/µl forms an elastic, cross-linked gel releasing growth factors. Macroscopic view on PRP-gels prepared from 315 µL PRP and activated with 5 U/ml thrombin. Scale bar depicts 30 mm.

Figure 2. Rheological analysis of PRP-gels. Storage (G') and loss (G") moduli as function of the angular frequency showing effective cross-linking of gels. (**A**) Influence of different platelet concentrations at a constant thrombin concentration of 5 U/ml on gel properties and (**B**) influence of different thrombin concentration on properties of PRP-gels with 2000x10^3 platelets/µl. Error bars represent SEM, $n \geq 3$

Figure 3. Growth factor release from different PRP-gels. Temporal (left) and cumulative (right) release of TGF-β1 (**A**), VEGF (**B**), PDGF-AB (**C**) and PDGF-BB (**D**) measured in the supernatants (PBS) of different PRP-gels using ELISA. Measurements were taken at indicated time points after gelification. Data is represented as mean SEM.

Figure 4. PRP-gels release inflammatory proteins. Supernatants from PRP-gels from 2 donors were collected after 12h of incubation and pooled and the relative abundance of inflammatory proteins was analyzed using an antibody protein array (**A**). Temporal (**B**) and cumulative (**C**) release of CCL5 from PRP-gels measured by ELISA. Error bars indicate SEM, n = 3

Figure 5. Proliferation and viability of MSCs in PRP-gels. (A) Proliferation of MSC incorporated in different PRP-gels assessed by DNA quantification. Error bars are SEM, n = 3. (**B**) Cells were enzymatically retrieved from PRP-gels, subjected to Propidium Iodide (PI) staining and cell viability analyzed using flow cytometry. Statistical significance at $p < 0.05$ (*) was determined using Kruskal-Wallis test. Error bars are SEM, n = 3. (**C**) Fluorescent microscope images of MSCs encapsulated in PRP gels after staining with calcein (green, living cells) and ethidium homodimer (red, dead cells). Scale bar represents 500 µm.

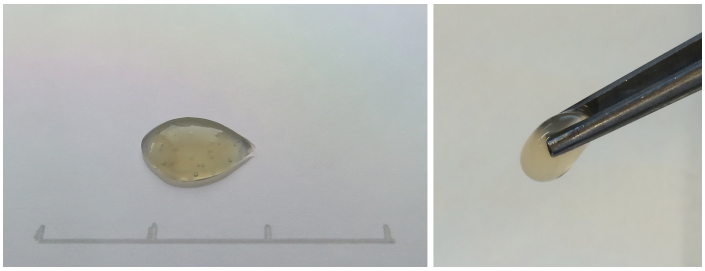

Fig. 1.

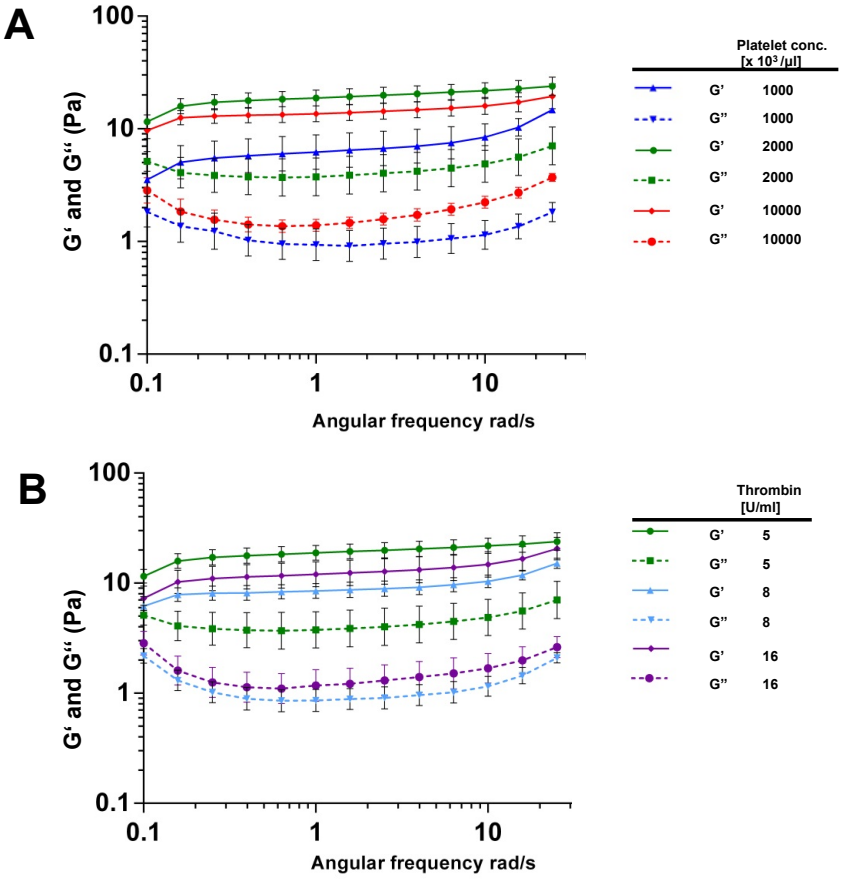

Fig.2.Rheological analysis of PRP-gels:

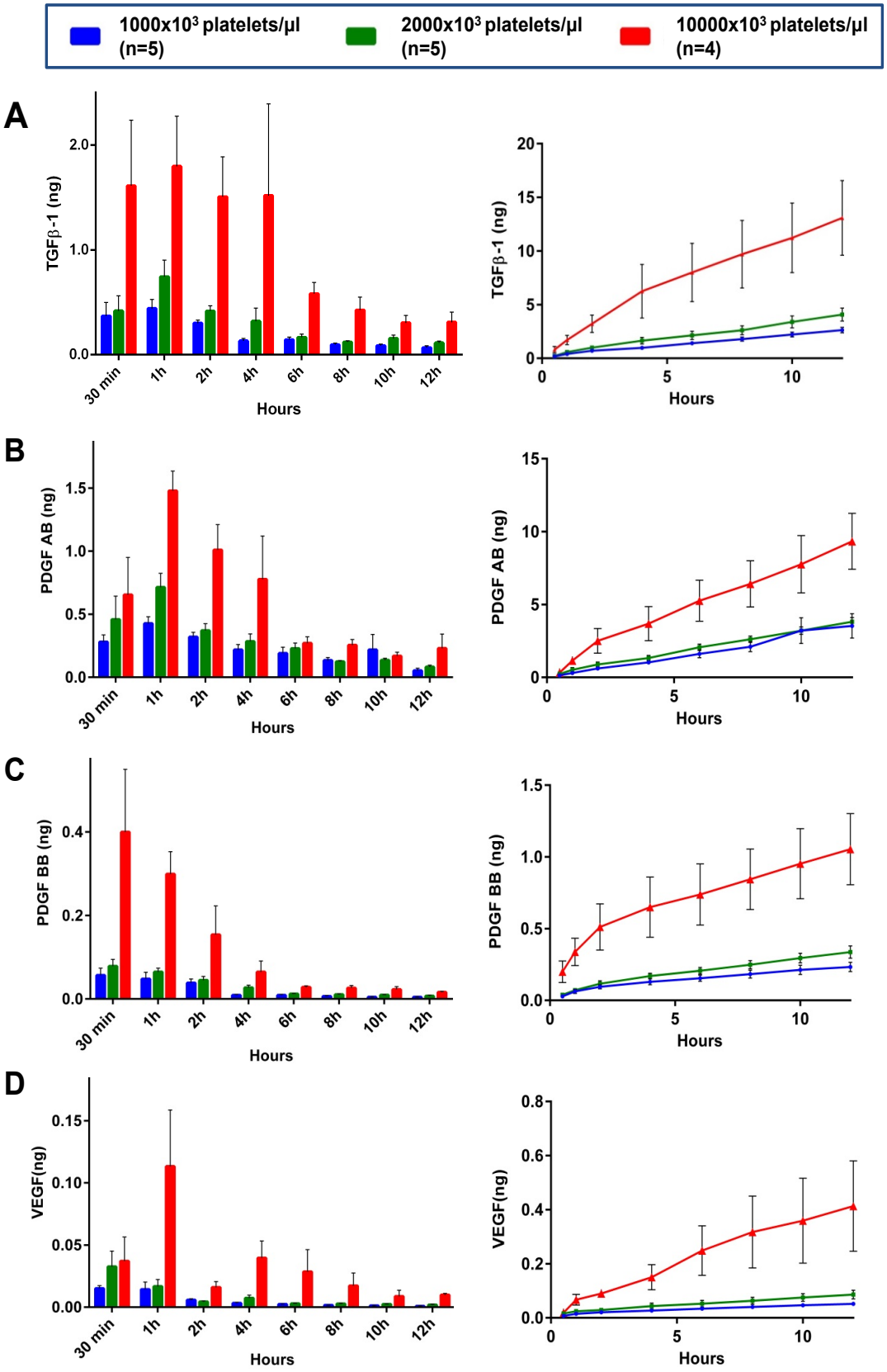

Fig. 3.Growth factor release from different PRP-gels.

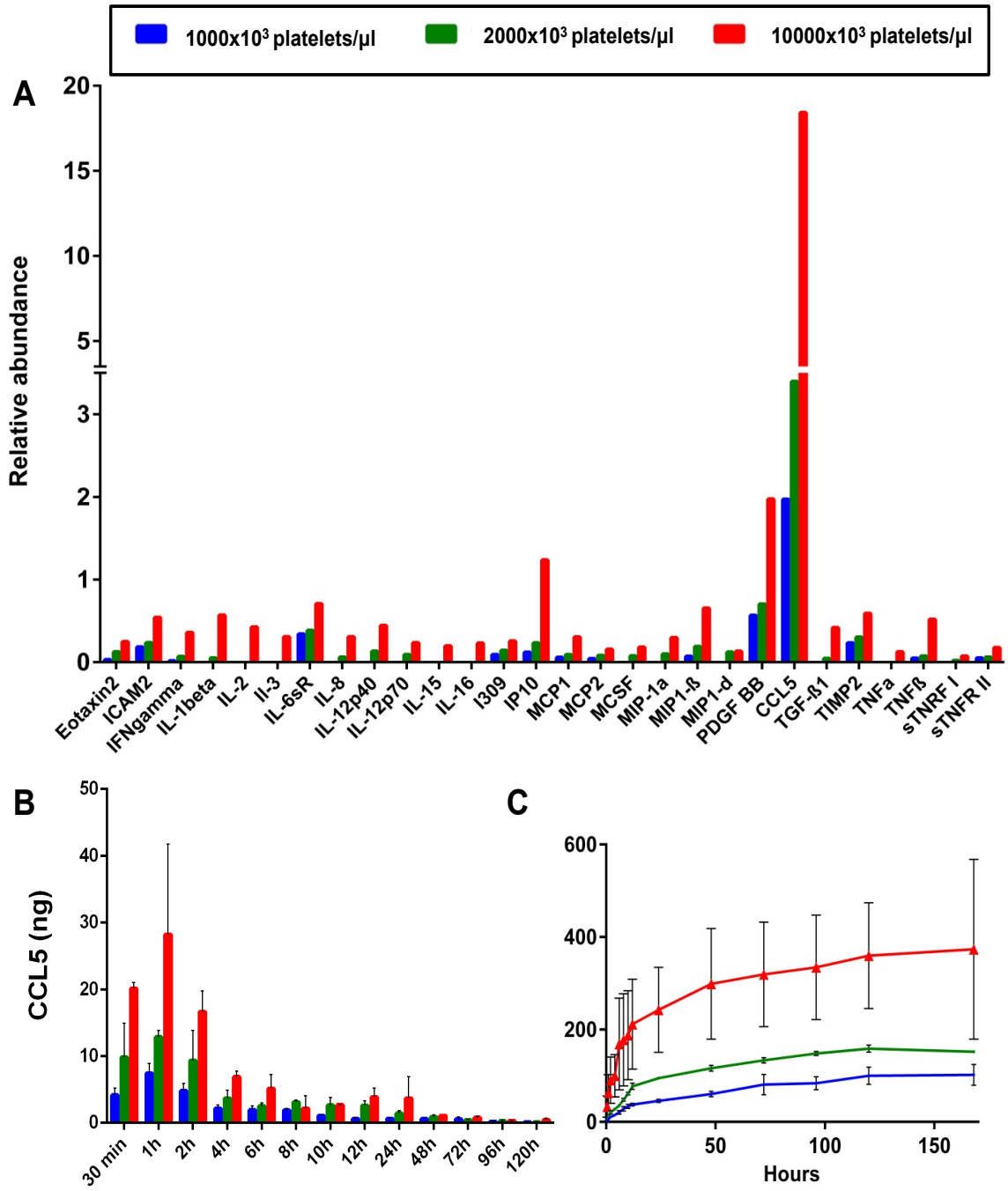

Fig.4. PRP-gel secretes inflammatory proteins.

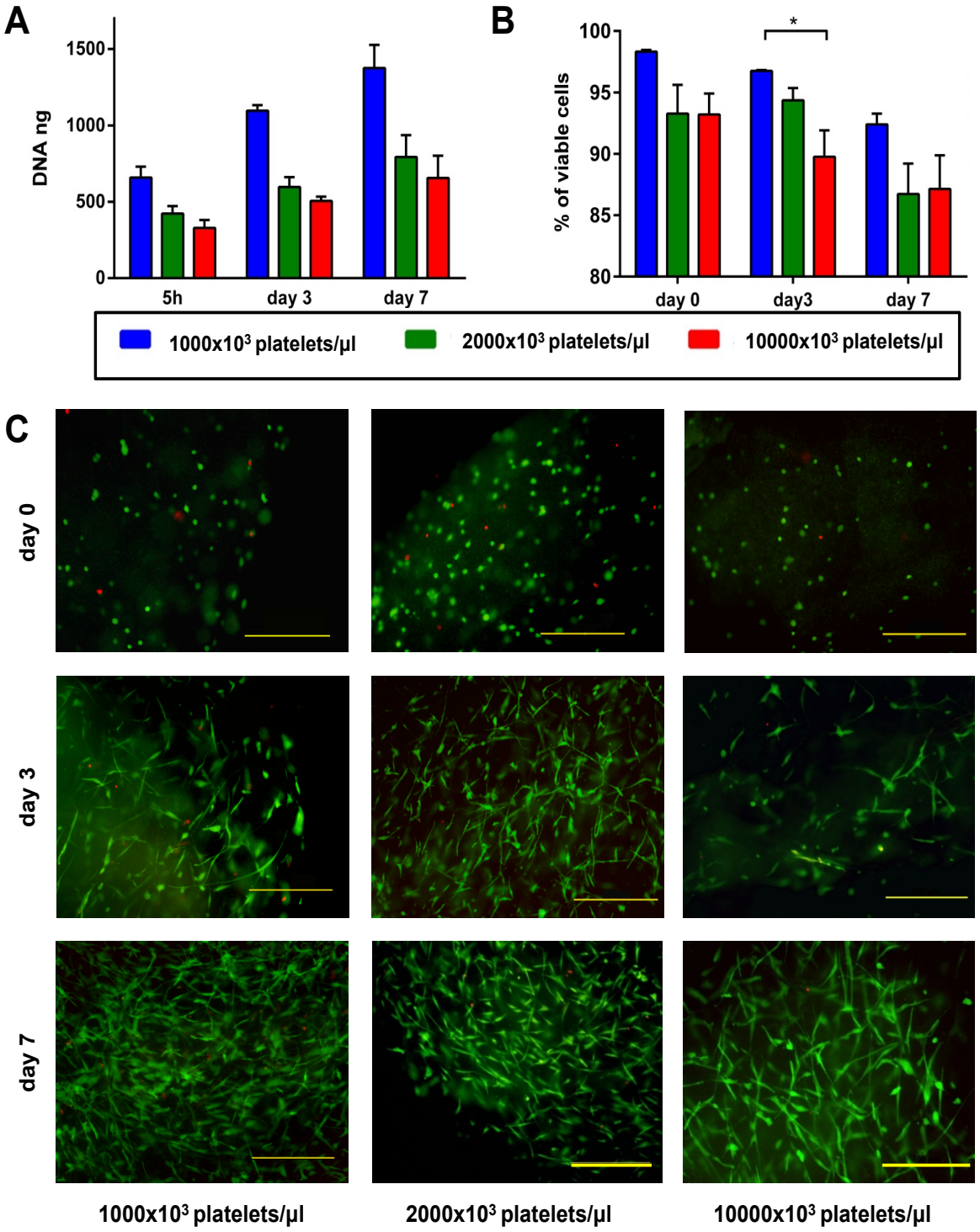

Fig.5. Proliferation and viability of MSCs in PRP-gels.

Lebenslauf/CV

Name	Jagoda Jalowiec
Geburtsdatum	07.02.1984
Geburtsort	Debica
Nationalität	Polin
Heimatort	Debica

1991 – 1999	Grundschule Debica, Polen
1999 – 2003	Allgemeinbildendes Lyzeum, Debica, Polen
2003 – 2007	Studium Tierzucht und Tierhaltung Fakultät für Biologie und Tierzucht, Naturwissenschaftliche Universität, Wroclaw, Polen
2005 – 2011	Studium Veterinärmedizin, Fakultät für Tiermedizin, Naturwissenschaftliche Universität, Wroclaw, Polen
März 2011	Staatsexamen, Dipl. Tierärztin, Fakultät für Tiermedizin, Naturwissenschaftliche Universität, Wroclaw, Polen
2014 – 2015	Anfertigung der Dissertation unter der Leitung von Prof. em. Dr. med. vet. Dr. h.c. Jörg Auer, Dipl. ACVS/ECVS, Klinik für Pferdechirurgie, Departement für Pferde, Vetsuisse-Fakultät Universität Zürich, Schweiz
2015 – dato	Intern, Department für Pferde, Vetsuisse-Fakultät Universität Zürich, Schweiz
2013 – 2014	Veterinary Research Fellow in Musculoskeletal Regenaration AO Foundation, Davos, Schweiz
2013	Assistenzärztin in der Pferdeklinik Ludwigshafen, Deutschland
2011 – 2012	Intern, Pferdeklinik,Veterinärmedizinische Universität Wien, Österreich